RETINAL DAMAGE FROM REPEATED SUBTHRESHOLD EXPOSURES USING A RUBY LASER PHOTOCOAGULATOR

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FOREWORD

This work was performed in the Oculo-Thermal Function, Ophthalmology Branch, under task No. 630105 between 2 April 1970 and 15 May 1970. The paper is a partial requirement for satisfactory completion of Phase II Residency in Aerospace Medicine. The paper was submitted for publication on 18 June 1970.

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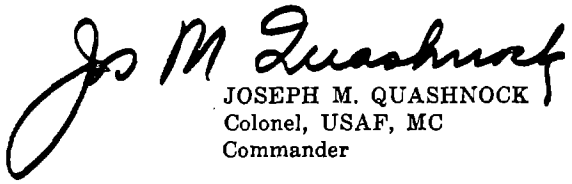
The animals involved in this study were maintained in accordance with the "Guide for Laboratory Animal Facilities and Care" as published by the National Academy of Sciences–National Research Council.

This report has been reviewed and is approved.



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ABSTRACT

A ruby laser photocoagulator was used to deliver single and multiple subthreshold exposures to the retina of *Macaca mulatta*. The subexposure parameters were 0.5-msec. pulse, 1.3-mm.-diameter focal spot, and energy density of about 375 mJ/cm.^{2} Single exposures produced no microscopic changes on sacrifice at 1 to 30 days following exposure. Repetitive exposures (7 to 17 exposures) at the same energy level invariably caused characteristic damage in the outer retinal layers. These cumulative effects are similar to and more widespread than the minimal-damage lesions produced by single suprathreshold exposures using higher energy density (900 to 1000 mJ/cm.^{2}). Current safety criteria are based on the assumption that laser damage occurs on an all-or-none basis so that damage is not cumulative if a single exposure causes no visible lesion. This work indicates that single subthreshold exposures which are less than half of a threshold dose are cumulative and therefore each "subthreshold" exposure must damage or in some manner increase the retinal susceptibility to subsequent exposures. Additional work must be done to determine the minimum amount of energy which will produce cumulative effects and how long the susceptibility persists after a single exposure.

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I. INTRODUCTION

Many of the biologic effects of laser energy remain unexplored 11 years after the first operating model was built by Maiman (1) in 1959. To cite some outstanding examples in the field of ophthalmology: No definite correlation exists between energy delivered per retinal spot size and the amount of functional decrement which ensues; there is no proof yet that delayed effects do not occur in a manner analogous to the way gamma rays cause subcellular changes; hazard evaluation literature usually contains the advice that a person who has had an ocular burn should seek a knowledgeable physician for appropriate treatment; yet no treatment for retinal burns is known (2).

Another unanswered question concerns the effect of repeated subthreshold exposures to the retina. A threshold exposure is one which causes a barely detectable lesion at some specific time interval after the trial burn. Threshold studies of the retina have used a number of different damage criteria, all of which depend on ophthalmoscopic or histologic evidence of altered tissue (3). Multiple subthreshold exposures are never considered a significant hazard in laser facility evaluations; yet there has been no evidence to support such confidence, and recent work done with conventional light sources indicates that irreversible retinal damage can occur following exposures to as little as 1000 ft.-c. of ordinary fluorescent light for one week (4). Multiple subthreshold accidental exposure is probably not a great hazard considering the possibility of having the laser focus on the same area by chance alone, especially if the focal spot is around 30μ in diameter. Subthreshold exposures thoughtlessly incurred by repeated direct viewing might cause damage to the fovea centralis, however, if such "doses" are indeed cumulative in man. Most laboratory workers are careless with laser beams "known" to cause no damage from a single exposure, and the same attitude is prevalent in industrial and field usage.

This study is a first attempt to determine whether or not laser exposures which are below microscopic threshold might be cumulative in an infrahuman primate *(Macaca mulatta)*.

II. MATERIALS AND METHODS

Selection of equipment and experimental design was arbitrary. The research goal was development of a technic suitable for testing the hypothesis that multiple subthreshold exposures would *not* cause cumulative damage. Although a number of technical problems arose, these were solved in the most expedient manner. Perhaps better solutions might be found by workers with a different range of alternatives.

In order to ensure repeated exposures to the same area, the spot size had to be relatively large. Trials using various lens systems to obtain a focal diameter of 0.7 mm. to 1.0 mm. were successful. The Krypton C-W laser available did not deliver sufficient power density to burn the retina even at 3-second exposures; therefore burn threshold for that system could not be determined.

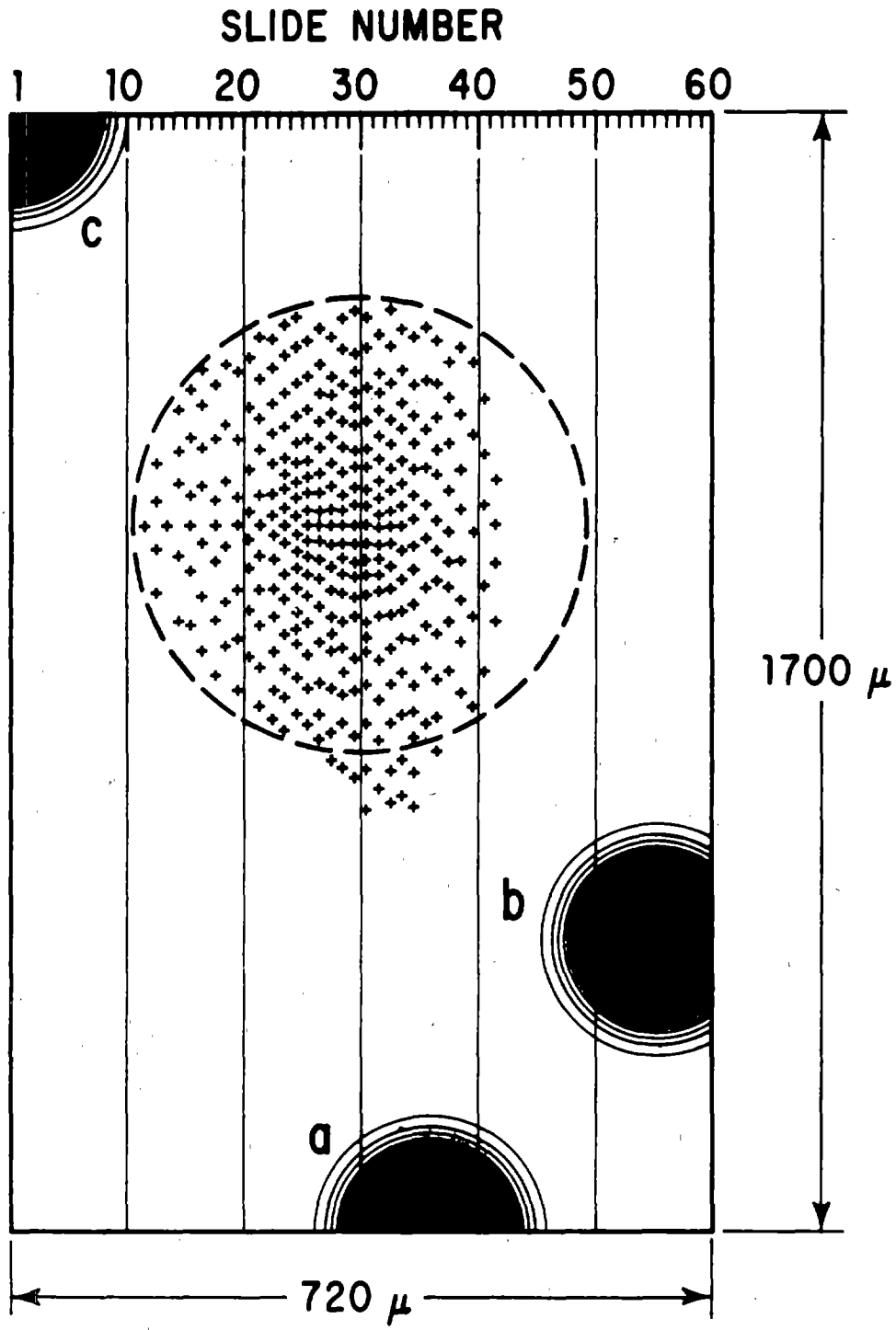

FIGURE 1

Effect of 10 subthreshold exposures (monkey 63T, path. No. A70-471). + = pathologic changes seen; ◌ = theoretical target area; a,b,c, *= marker burns.*

It was expected that subthreshold doses would not be cumulative but, if they were, that the doses should be reasonably close to threshold to allow cumulative effect to be apparent in a reasonable number of exposures. If ten doses at about 10% threshold were 100% cumulative, an effect would be barely detectable. Partly because of the capabilities of the laser used and in hopes of demonstrating something significant, it was decided to try for exposures which would be a little less than 50% of threshold and deliver at least five to ten such doses.

A laser photocoagulator (Optics Technology Inc., model M-10, Mark II) was available and had the desirable feature of adjustable spot size (five positions ranging from 2.0° to 5.5° beam divergence). Beam focal size was measured on graph paper after being passed through lenses simulating the monkey's optical tract and was found to be 0.47 mm. to 1.3 mm., respectively. The smallest spot was used for marker burns to permit easy localization of target areas and to allow appropriate tissue sections to be cut and oriented at both gross and microscopic examination of the desired target area.

The laser output was calibrated with an Eppley thermopile, model 100 (200.3 μv./J), while a portion of the beam was split off and recorded on a Moseley 68M strip-chart recorder. The laser was designed for clinical purposes; therefore in normal usage a burn would be sought every time. A 0.5 OD neutral density filter was inserted in the beam path to ensure uniform lasing action at the low triggering energies required. The laser specifications were: maximum output, 250 mJ at 694.3 nm.; 0.2-msec. pulsewidth; and variable beam divergence as previously noted. The power output was controlled by adjustment of an arbitrary "power unit" scale and was continuously adjustable for outputs of 2 mJ to 25 mJ for this experiment.

Macaca mulatta, weighing 2 to 3 kg. each, were given 1% atropine sulfate in each eye at 16 hours and 2 hours prior to laser exposure. Sernylan (0.5 mg./kg.) was administered intramuscularly to tranquilize the animals before insertion of an intravenous catheter. Pentobarbital sodium (25 mg./kg.) was titered to sedate them and control eye movements. After the animals were sedated, the eyes were refracted; only monkeys which were within ½ diopter of emmetropia were accepted in the study.

Control and experimental target areas were restricted to the paramacular region within a 3-mm. radius of the fovea and only on the temporal side of each eye since there is evidence to show that this would assure a reasonable degree of uniformity for threshold doses (5). After a target area was selected, small-spot marker burns were placed to each side, about 2-mm. apart. These retinal marks were visible immediately and were also visible at dissection of the eye as 0.5-mm. pale grey spots. Corneas were kept moist by frequent lid closure; when pauses of more than 2 minutes were anticipated, the eyes were patched.

A time-consuming but technically excellent histologic method had already been developed for the study of minimal lesions in the primate retina (6). In effect, the eye was partially fixed by upper body perfusion before any tissue degeneration occurred and before enucleation was begun. Retinal detachment was therefore no problem, and the tissue blocks were imbedded in plastic (Epon) which permitted serial sections 2μ thick to be examined. Paragon and osmium were used to stain the sections. The quality of the finished slides exceeded that of the usual methods described and pictured in the literature. After the target area was defined, 450 to 650 serial sections were examined, and a map was drawn of the markers and experimental exposure site (fig. 1). After the landmarks were established scanning at low power, the target areas were reviewed for signs of tissue damage at 430× and 970×. There were single subthreshold exposure controls for each of the multiple exposure target areas, and these were reviewed very carefully to be sure that no minimal damage had occurred. Exposure dosage and time interval from exposure to animal sacrifice were varied to obtain some

TABLE I

Multiple subthreshold exposures

Monkey eye	Number of exposures	Exposure to sacrifice (days)	Energy density (mJ/cm.^{2})	Result (degree and extent)
33 OS	7	1	263-377	Minimal and >100 cells
63 OD	10	1	338-414	Moderate and >100 cells
19 OS	9	14	271-421	Moderate and >200 cells
29 OS	17	30	377-564	Moderate and >500 cells

TABLE II

Single exposures, near and subthreshold

Monkey eye	Number of exposures	Exposure to sacrifice (days)	Energy density (mJ/cm.^{2})	Result (degree and extent)
33 OS	1	1	865	No change
33 OD_a	1	5	1579	Minimal and >20 cells
33 OD_b	1	5	338	No change
63 OD	1	1	377	No change
19 OS	1	14	346	No change
29 OS	1	30	377	Minimal and 2 cells

idea of the limits of response. These parameters will be presented in the next section along with the results.

III. RESULTS

Data are presented on five eyes from four monkeys. The other three eyes were not included for the following reasons: one was used for establishing the near-threshold dose for lesions visible with the ophthalmoscope at 24 hours; one was a histologic control; and the third was sectioned by a person unfamiliar with the technic and the markers, and hence the orientation was lost. In considering the data in tables I and II, it must be remembered that time did not permit completion of an extensive protocol and that the study was intended to be only an investigation into means.

The time interval between each of the multiple exposures was 1 to 3 minutes for all eyes. The thermal relaxation period of the retina is certainly much shorter than this, but longer time intervals should also be tried in the future. The variation was due to the difficulty in finding the target area with a good field of vision and a moist cornea to focus through at exactly the same time interval.

The altered appearance of the outer retinal layers and pigment epithelium is clearly shown

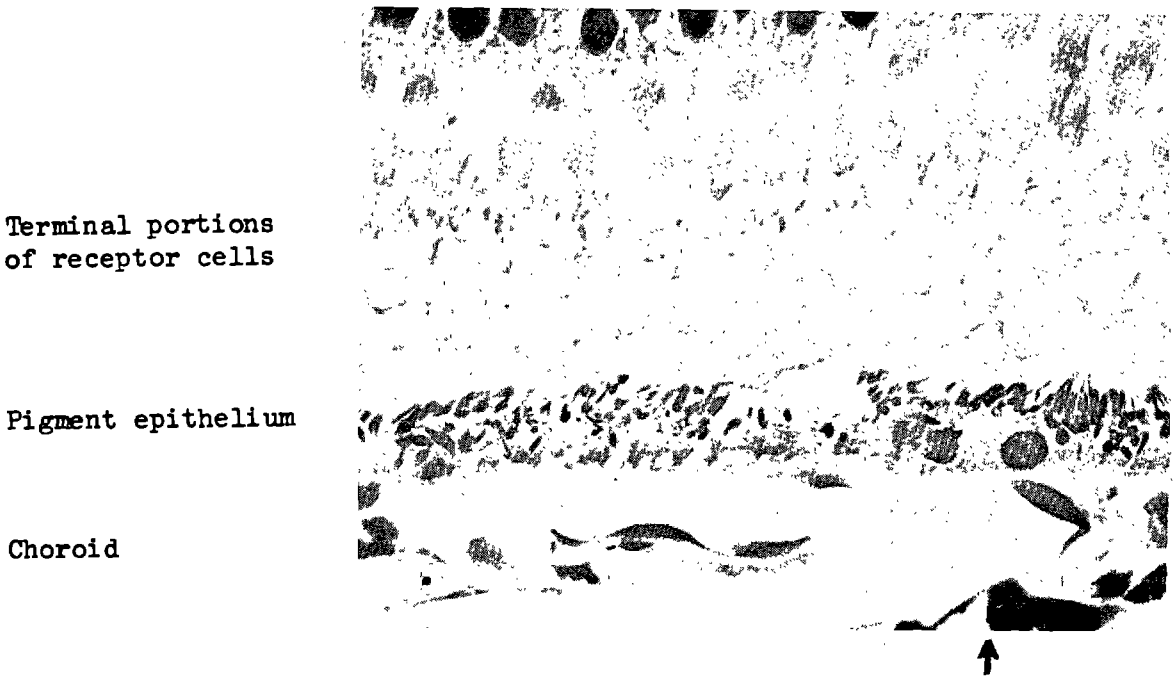

FIGURE 2

Monkey eye 33 OD_a 1579 mJ/cm.²—Note small vacuoles along Bruch's membrane and larger one above epithelial cell. Pigment granules are disrupted. The tissue to the right of the arrow is normal. (24 hours postexposure) 970×

in the photographs. Figure 2 shows a minimal lesion in a 5μ section prepared in paraffin and stained with hematoxylin and eosin (the only such preparation in the study). This is comparable to what other investigators have shown in the literature as equivalent to a minimal lesion. The subject (33 OD_a in table II) received a single exposure of 1579 mJ/cm.² No ophthalmoscopic change was evident at 2 hours after exposure, but a faint lesion was present after 24 hours. The highest energy density given following which no microscopic change could be detected was 865 mJ/cm.² (33 OS in table II—an exception to this is 29 OS). No photographs of completely normal tissue are shown, but the left-hand side of figure 3 is essentially normal. The right-hand side shows one half of a typical marker burn. Figure 4 is a higher magnification of the edge of the same marker burn seen in figure 3, and the center third of figure 4 is a typical representation of moderate retinal changes following multiple exposure. Minimal changes are seen in figure 5. Notice the disrupted pigment granules, especially the absence of some of the fusiform granules normally seen interdigitating with the terminal ends of the rods and cones. An amorphous material is present, which might represent edema along the inner surface of the pigment epithelial cells. Although Bruch's membrane remains intact in all but intense marker burns, it becomes thickened and clumps of vacuoles appear along it. Small ones are seen in figure 5 which is one view of 33 OS (table I). Older lesions show pigment-laden macrophages and occasional pyknotic nuclei in both the inner and outer nuclear layers. The only change noted following a single exposure *below* threshold occurred in 29 OS (table II). The change occurred in two adjacent pigment epithelial cells, which were mildly vacuolated and showed loss of some pigment. The degree and extent of the "lesion" were not as severe as the changes shown in figure 5, and it is tempting to suggest that these very minimal changes are what one can expect to see 30 days

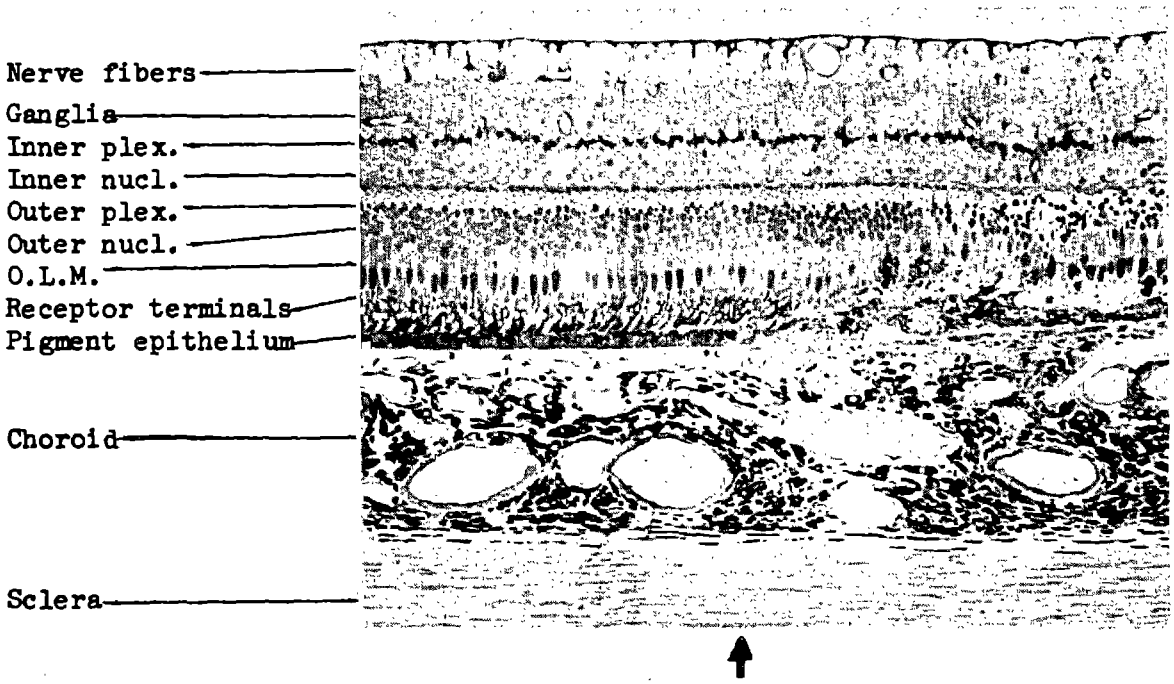

FIGURE 3

Monkey eye 33 OS, 83 J/cm.^{2} marker—The area in the center of the photograph is used for figure 4. This view shows half of a typical marker measuring about 450μ in diameter. The marker is to the right of the arrow. 100×

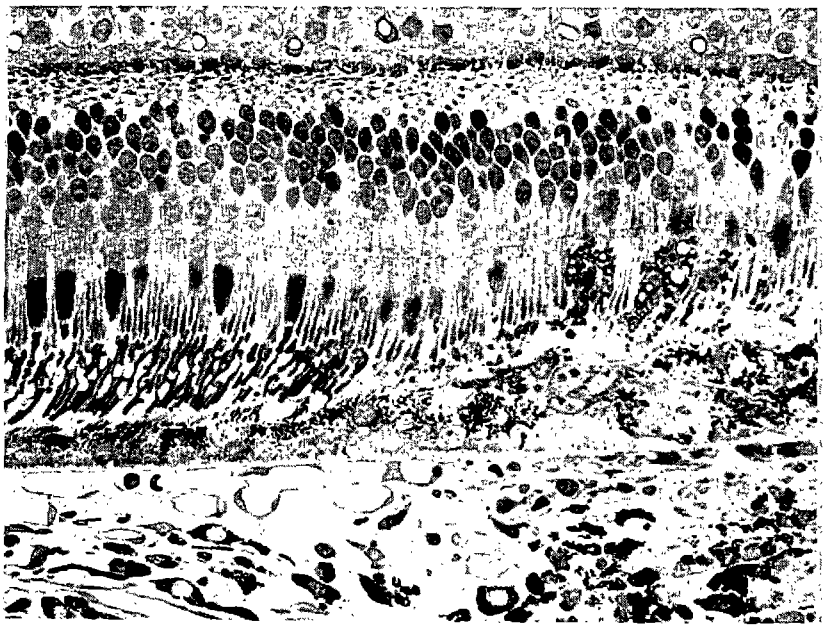

FIGURE 4

Monkey eye 33 OS, 83 J/cm.^{2} marker (margin)—The center of this photograph is typical of "moderate" changes seen in cumulative doses as shown in table I. 430×

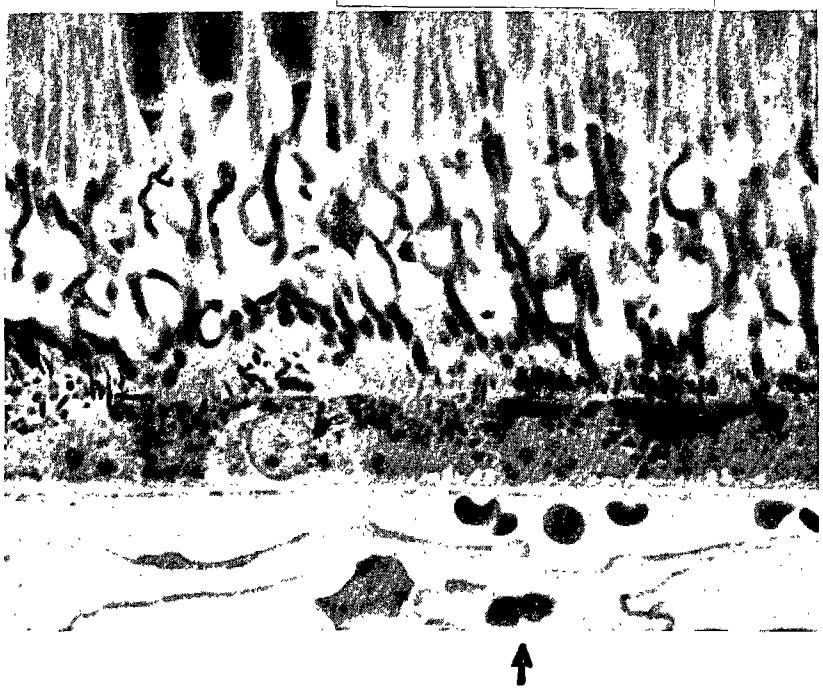

FIGURE 5

Monkey eye 33 OS, 7 exposures of 263 to 377 mJ/cm.²—The area to the left of the arrow shows typical cumulative damage of minimal degree. The tissue to the right is normal. 970×

after exposure to a 50% dose. The absence of widespread changes makes it unlike the minimal change pattern that was seen in suprathreshold large spots or in the cumulative damage target areas.

A comparison of figures 2 and 5 clearly shows the advantages of 2μ sections, stained with osmium and paragon.

IV. DISCUSSION

The ruby photocoagulator was an excellent tool for this study in many respects. Its good features were variable spot size, hand-held laser head, accurate targeting, and ample energy. Drawbacks were nonhomogeneity of beam and variation in energy output. Neither of these two factors is a problem in clinical usage but both make accurate experimental work difficult. A shuttered C-W laser would avoid both these problems but at considerable cost of convenience to the operator.

Tissue preparation and slide staining are time consuming, but high-quality slides are necessary for detecting minimal lesions. Markers of 470μ diameter placed 2-mm. apart leave about 1500μ of normal tissue for use as a target area, and these markers remain the same diameter over a 30-day period.

Despite the limited amount of data, some conclusions can be drawn. Microscopic damage did not seem to regress with time. The older the lesion was, the more easily apparent it became. There was no evidence of repair in the sense of return to normal tissue and function. Scar tissue would probably be the end result of both markers and the moderately damaged areas. The threshold may have been too low (or high), but there is surprisingly close agreement with Ham's figure of 850 mJ/cm.² for paramacular large-spot ruby threshold (7). Whatever the threshold may have been for a given eye, the single subthreshold exposures caused no damage (or very

slight damage in the case of 29 OS), whereas the multiple subthreshold exposures uniformly caused a widespread characteristic lesion similar in degree to near-threshold single burns. If "hot spots" in the beam caused changes in the multiple exposure group, there should have been more evidence of the same event occurring in the single control exposures.

Future experimentation of this type should be directed to determining the safe interval between subthreshold exposures to avoid cumulative effects and the smallest subthreshold exposure which can still be cumulative.

No effort was made to determine mechanism of cumulative effect. Massive vitamin A release by light-destroyed receptors has been proposed and could explain the lesions seen (8). This would be another profitable line of inquiry and might even lead to a burn therapy rationale.

For a 1- to 3-minute repetition rate, ruby laser exposures which are half of a retinal threshold dose are cumulative after 7 to 17 subthreshold exposures. The cumulative damage does not appear to be reversible in 30 days.

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13. ABSTRACT

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